Biosynthesis of Xenovulene A®: formation of a cyclopentenone *via* a unique ring expansion–ring contraction mechanism

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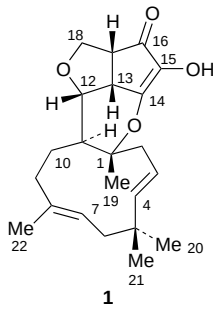
Incorporation studies with ^{13}C-labelled acetates and methionine in *Acremonium strictum* indicate that the cyclopentenone moiety in Xenovulene A® is formed *via* a unique pathway in which a C-methylated phenolic precursor undergoes ring expansion to a tropolone followed by two successive ring contractions resulting in incorporation of the C-methyl carbon into the five-membered ring.

Xenovulene A® **1**, a novel inhibitor of benzodiazepine binding to the GABA-benzodiazepine receptor,[1] was isolated from cultures of *Acremonium strictum*.[2] The structure of **1** contains an unusual furocyclopentenone moiety fused to a humulene-derived 11-membered ring. A number of minor co-metabolites of **1** have been isolated[3] in which the cyclopentenone moiety is replaced by a trioxygenated benzene **2** or highly oxygenated tropolone rings, **3** and **4**. While fungal metabolites containing all of these types of rings are known, the presence of all three in a single family is unique.



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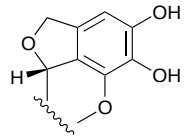

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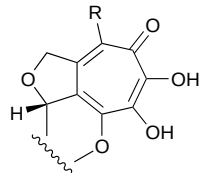


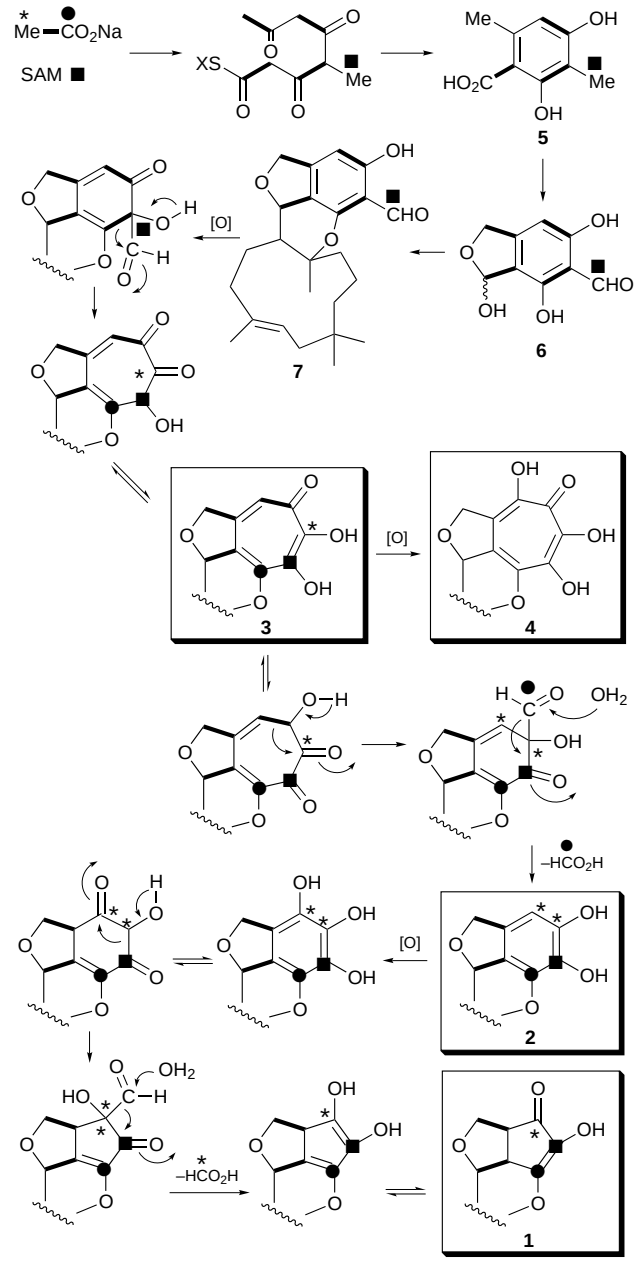
3 R = H
4 R = OH

Recently, two very similar fungal metabolites, pycnidione[4] and eupenifeldin[5] were isolated. These both contain a similar fusion of a tropolone to humulene. The higher plant metabolite, lucidene, also contains a humulene ring fused to an *o*-hydrobenzyl moiety.[6] The fungal tropolone, stipitatonic acid is derived from 3-methylorsellinic acid **5** with incorporation of the methionine-derived 3-methyl group into the polyketide-derived aromatic ring.[7] In addition, the fungal cyclopentenone metabolites, terrein[8] and cryptosporiopsonol[9] have been shown to be derived by ring contraction of the aromatic ring of dihydroisocoumarin intermediates. Thus a plausible pathway to the Xenovulene metabolites **1**–**4** appeared to be *via* the polyketide-derived 3-methylorsellinic acid **5** (Scheme 1) which would be converted by standard biosynthetic modifications to the lactol **6**. This would react with the 6,7 double bond of humulene, either *via* a carbocation mediated addition or cycloaddition of the readily derived quinomethane to give the key tetracyclic intermediate **7**.

Ring expansion of the phenolic ring in **7** followed by hydroxylation would give the tropolones **3** and **4**, whereas oxidative decarboxylation of **7** would give **2**, ring contraction and decarboxylation of which would lead to Xenovulene A® **1**.

We now report incorporation studies with ^{13}C-labelled acetates and methionine which render this branched pathway untenable and indicate a linear relationship among the metabolites.

The results of incorporations† of [1-^{13}C]-, [2-^{13}C]- and [1,2-$^{13}C_2$]-acetates and [*methyl*-^{13}C]methionine into Xenovulene A® are summarised in Fig. 1. Surprisingly, no intact acetate unit was present in the cyclopentenone ring and C-15 was not



Scheme 1

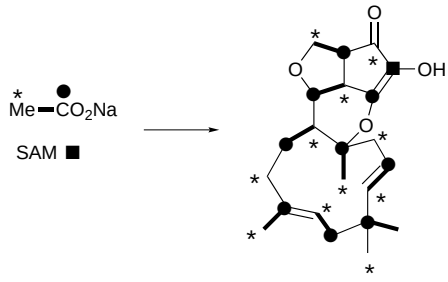


Fig. 1

derived from either the methyl or carboxy carbons of acetate. This was accounted for on feeding ^{13}C-labelled methionine which gave an approximately 8% enrichment at C-15. To accommodate these results, the remarkable sequence outlined in Scheme 1 is proposed. Hydroxylation of **7** allows ring expansion *via* an α-ketol rearrangement to give **3** which, after further tautomeric adjustment, can ring contract and lose formic acid to form the trioxygenated benzenoid metabolite **2** with incorporation of the methionine-derived carbon into the aromatic ring. Further oxidation, α-ketol mediated ring contraction and loss of formate generates Xenovulene A® with the observed labelling pattern.

Work is in progress to obtain further information on the detailed mechanisms and the exact oxidation levels of the intermediates on the pathway. For example, analogous pathways in which the formyl group in **7** is at the hydroxymethyl or carboxy level can be proposed, but these necessitate more extensive reductive and oxidative adjustments to obtain the final structures. Overall, this represents a remarkable and unprecedented pathway in which a cyclopentenone is formed from a phenolic precursor *via* one ring expansion and two successive ring contractions.

Footnotes and References

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† Precursors were fed to shake flask cultures of *A. strictum* at levels of 1.0 g l^{-1} ([1-^{13}C]- and [2-^{13}C]-acetates) or 0.5 g l^{-1} ([1,2-$^{13}C_2$]acetate and [*methyl*-^{13}C]methionine). Xenovulene A® (approximately 10 mg l^{-1}) was purified by flash chromatography followed by reversed phase HPLC. Incorporation of singly ^{13}C-labelled acetates resulted in enrichments of 1–3 atom%. No significant differences in level of labelling were observed between the polyketide and terpenoid moieties. The minor metabolites **2**–**4** were not isolated.

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